Thrombophilia ad dies vitae

Cohn, D.M.

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Chapter 9

Common genetic variation at the Endothelial Lipase (LIPG) locus and the risk of coronary artery disease and deep venous thrombosis

Vergeer M, Cohn DM, Boekholdt SM, Sandhu MS, Prins H, Ricketts SL, Wareham, NJ, Kastelein JJP, Khaw KT, Kamphuisen PW, Dallinga-Thie GM
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Abstract

Background

Low levels of high-density lipoprotein cholesterol (HDL-C) are a risk factor for coronary artery disease (CAD) and possibly for deep venous thrombosis (DVT). Endothelial lipase is involved in HDL-C metabolism. Common variants in the endothelial lipase gene (*LIPG*) have been reported to be associated with HDL-C levels and atherothrombosis, but these findings were not consistent.

Objectives

We determined whether 5 tagging single nucleotide polymorphisms (SNP) in *LIPG* were associated with lipid parameters, the risk of CAD and the risk of DVT.

Methods

We used the prospective case-control study nested in the EPIC-Norfolk cohort (1138 CAD cases, 2237 matched controls) for initial association studies and, subsequently, the ACT study (185 patients with documented DVT, 586 patients in which DVT was ruled out) to replicate our findings regarding DVT risk.

Results

In EPIC-Norfolk, we found that the minor allele of one SNP, rs2000813 (p.T111I), was associated with moderately higher HDL-C and apolipoprotein A-I levels, higher HDL particle number and larger HDL size. No variants were associated with CAD risk, but 3 variants were associated with DVT risk (odds ratios 0.60 (95%CI 0.43-0.84), 2.04 (95%CI 1.40-2.98) and 1.67 (95%CI 1.18-2.38) per minor allele for rs2000813, rs6507931 and rs2097055 respectively, $p<0.005$ for each). However, the association between *LIPG* SNPs and DVT risk could not be replicated in the ACT study.

Conclusion

Our data support a modest association between the *LIPG* rs2000813 variant and parameters of HDL metabolism, but no association between common genetic variants in *LIPG* and CAD or DVT risk.

Introduction

Plasma high-density lipoprotein cholesterol (HDL-C) levels correlate inversely with the risk of coronary artery disease (CAD)[1] and are also suggested to be associated with venous thromboembolism.[2-4] Family studies underline that 40-60% of the variation in HDL-C levels is explained by genetic factors[5] and many candidate genes involved in human HDL metabolism have been identified.[6] Endothelial lipase constitutes such a candidate and is a member of the lipase family. These lipolytic enzymes are involved in lipid absorption, transport, and metabolism. Endothelial lipase is synthesized in endothelial cells and possesses phospholipase activity preferentially directed at HDL phospholipids.[7] Overexpression of endothelial lipase reduces HDL-C, whereas deficiency of endothelial lipase leads to elevation of HDL-C levels in mice.[7-9] In humans, high levels of endothelial lipase are significantly associated with features of the metabolic syndrome and with coronary artery calcification.[10]

Genetic association studies have yielded conflicting results with respect to associations between common genetic variants in *LIPG* and HDL-C levels.[9,11-18] However, in several genome-wide association studies single nucleotide polymorphisms (SNPs) near *LIPG* were identified as being associated with HDL-C.[19-24] Furthermore, rare loss-of-function variants in *LIPG* were recently shown to be a cause of elevated HDL-C in humans.[25]

The data regarding associations between *LIPG* gene variants and CAD risk are mixed, however. The minor allele of a common variant resulting in a Thr to Ile substitution at codon 111 (rs2000813), was found to occur less frequently in patients who had a history of myocardial infarction compared with those without.[13] Similar findings were reported from two small case-control studies.[15,16] Furthermore, the same SNP has been reported to be associated with atherothrombotic cerebral infarction in Japanese women.[26] However, in a recent large investigation in three independent prospective cohorts by Jensen et al, no association between this SNP and the risk of CAD was found.[18]

The association between genetic variation in *LIPG* and the occurrence of deep venous thrombosis (DVT) has, to our knowledge, never been specifically addressed. Since the potential interrelation between endothelial lipase, high-density lipoprotein metabolism and (athero)thrombosis is currently unclear, we aimed to evaluate whether common genetic variants in *LIPG* associate with plasma lipid parameters, CAD or DVT risk.

Methods

Studies were approved by Institutional Review Boards and conducted according to the Declaration of Helsinki. Written informed consent was obtained from all participants.

Data sources

The EPIC (European Prospective Investigation into Cancer and Nutrition)-Norfolk cohort consists of a prospective population of 25,663 men and women between ages 45 and 79. EPIC-Norfolk is part of the 10-country collaborative EPIC study designed to investigate determinants of cancer. From the outset, additional data were obtained in EPIC-Norfolk to enable the assessment of determinants of other diseases, including self-reported history of DVT at baseline and prospective data on the occurrence of CAD. We performed analyses in a subset of this cohort, a case-control cohort consisting of men and women who developed fatal or nonfatal CAD during 7 years of follow-up (n=1138), and controls, matched for age, sex, and enrolment time (n=2237). CAD was defined as codes 410 to 414 according to the International Classification of Diseases, Ninth Revision. Participants were identified as having CAD during follow-up if they had had a hospital admission or had died with CAD as the underlying cause. Previous validation studies in our cohort indicate high specificity of such case ascertainment.[27] To replicate the findings from this study, we genotyped all tagSNPs in the Amsterdam Case-control Thrombophilia (ACT) study, consisting of 771 outpatients who were referred to our hospital for evaluation of a suspected deep venous thrombosis (DVT).[28] In this study, 185 patients with objectively confirmed first DVT were compared to 586 patients in whom this diagnosis was ruled out.

Laboratory analyses

Genotyping

We used the HAPMAP database and the TAGGER algorithm to capture most of the common variation in the *LIPG* locus (NM_006033). For an in-depth discussion of this method, we refer to reference [29]. Briefly, we selected from HAPMAP all common genetic variants (minor allele frequency > 0.1) in the *LIPG* locus in a population of European ancestry. However, because of high correlations (high r^2) among some pairs of SNPs, genotyping both SNPs would offer only limited extra information. A tagging SNP approach uses the knowledge of associations between genetic variants (linkage disequilibrium [LD] structure) to limit the number of SNPs that needs to be genotyped. TagSNPs are those SNPs which most effectively represent (or 'tag') all the SNPs in a particular locus. We selected

tagSNPs using an r^2 cut-off level > 0.8. For the EPIC-Norfolk study genotyping was conducted by KBioscience (http://www.kbioscience.co.uk) using KASPar technology. Genotyping of SNPs in the other cohort was carried out on an ABI 7900 system, using Assay by Design™ assays (Applied Biosystems, Foster City, CA, USA).

Biochemical analyses

Total cholesterol, HDL cholesterol and triglycerides were determined using standard laboratory procedures within one hour after (non-fasting) blood sampling. Low-density lipoprotein LDL cholesterol levels were calculated with the Friedewald formula. Serum levels of apolipoprotein A-I (apoA-I) and B (apoB) were measured by rate immunonephelometry (Behring Nephelometer BNII, Marburg, Germany) with calibration traceable to the International Federation of Clinical Chemistry primary standards. The interassay coefficient of variation (CV) of the apoA-I and apoB measurements were 5% and 3%, respectively. Serum concentrations of apolipoprotein A-II (apoA-II) were measured with a commercially available immunoturbidimetric assay (Wako Pure Chemicals Industries, Ltd, Osaka, Japan) on a Cobas-Mira autoanalyzer (Roche, Basel, Switzerland). The intra-assay and interassay CVs for this assay were 2.5% and 3.1%, respectively. HDL particle number and HDL size were measured with an automated nuclear magnetic resonance (NMR) spectroscopic assay as described previously.[30] In brief, particle concentrations of lipoprotein subclasses of different size were obtained directly from the measured amplitudes of their spectroscopically distinct lipid methyl group NMR signals. Summation of the HDL subclass levels provides total HDL particle concentration. For the present study, we grouped HDL subclasses as follows: small HDL (7.3 nm to 8.2 nm), medium HDL (8.2 to 8.8 nm), and large HDL (8.8 to 13 nm). NMR spectroscopy–measured HDL size was calculated as the mass-weighted average diameter of the HDL particles in a particular plasma sample. Plasma concentrations of C-reactive protein (CRP) were measured with a sandwich-type enzyme-linked immunosorbent assay as previously described.[31] Samples were analyzed in random order to avoid systematic bias. Researchers and laboratory personnel had no access to identifiable information and could identify samples by number only.

Statistical analyses

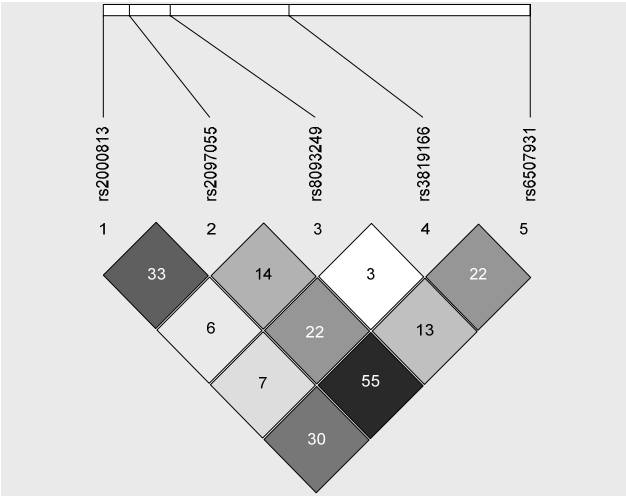
All statistical analyses were performed in cases with complete data, using SPSS version 16.0.2. Two-sided probability values of less than 0.05 were considered statistically

significant. Effects of SNPs on continuous variables were examined by ANOVA. To estimate the relative risk of CAD in EPIC-Norfolk, conditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals. Conditional logistic regression took into account the matching for sex, age and enrolment time, and was adjusted for Framingham risk score. We furthermore performed a meta-analysis on the relationship between rs2000813 and cardiovascular risk using our own data as well as the published data by Jensen et al.[18] To estimate the relative risk of DVT in EPIC-Norfolk and in the ACT study, differences between cases and controls were analysed by standard contingency table analysis using two-tailed chi-square test probabilities; linearity of this relationship was assessed using logistic regression analysis. LD plots were created with Haploview, version 4.1.[32]

Results

Using HAPMAP, we selected five *LIPG* tagSNPs. These tagSNPs were rs2097055 (c.460-320T>CT>C), rs8093249 (c.571+1480A>C), rs2000813 (c.332C>T; p.T111I), rs3819166 (c.793+142A>G) and rs6507931 (c.1377-108C>T). LD plots for the five tagSNPs in EPIC-Norfolk are displayed in Figure 1. The SNPs were not in LD as all r^2 values were below 0.8, confirming their non-redundant status as tagSNPs. All SNPs were in Hardy-Weinberg equilibrium (data not shown).

Figure 1. LD plot of *LIPG* tagSNPs



The relationships between the five selected *LIPG* tagSNPs are shown in an LD plot. The values in the plots represent r^2 between the appropriate SNPs ($\cdot 10^{-2}$) and were calculated using Haploview. All are below 0.8, confirming their non-redundancy.

Common genetic variants in LIPG and lipid levels

Of the five tagSNPs that were genotyped, only rs2000813 (p.T111I) showed a consistent relationship with HDL parameters. Characteristics of patients stratified according to T111I genotype are given in Table 1; characteristics of patients according to the genotype of the other four SNPs are presented in Supplementary Tables 1-4. Carriers of the I allele had higher HDL-C (0.034 mmol/L (95%CI 0.012-0.055) per allele, p=0.002), higher apoA-I levels (3.16 mg/dL (95%CI 1.48-4.85) per allele, p<0.0005), a larger HDL size as measured by NMR spectroscopy (40 pm (95%CI 15-65) per allele, p=0.002) as well as by gradient gel electrophoresis (33 pm (95%CI 10-56) per allele, p=0.005) and a higher concentration of HDL particles (0.34 nmol/L (95%CI 0.04-0.64) per allele, p=0.026). Body mass index, waist circumference, history of diabetes mellitus, blood pressure and CRP levels did not differ among carriers of different alleles. Total cholesterol, LDL cholesterol or apolipoprotein B levels were also similar across genotypes.

Table 1. Characteristics of individuals according to *LIPG*T111I genotype in EPIC-Norfolk

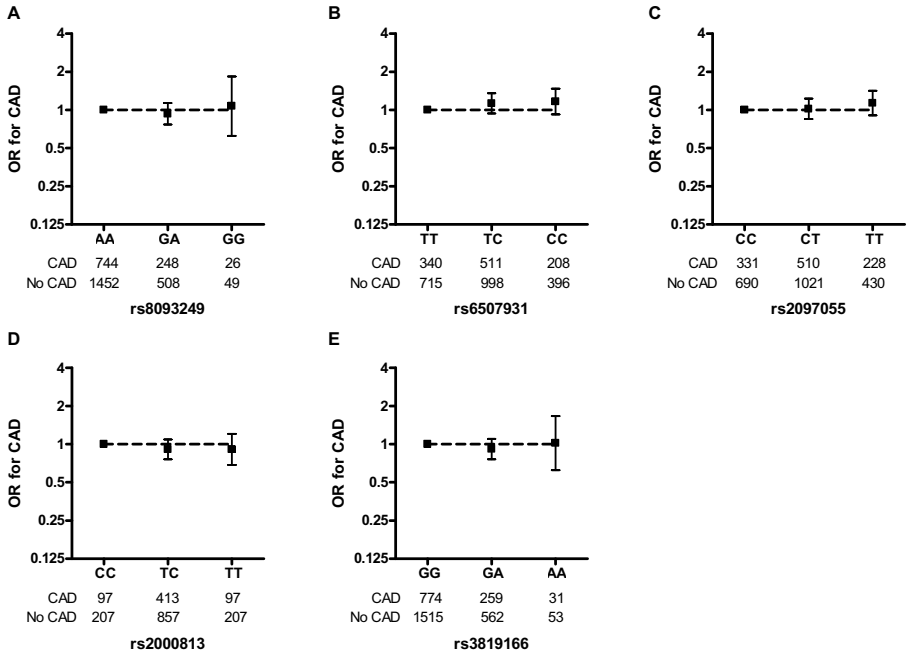
	rs2000813 (p.T111I)			
	CC	CT	TT	P
N	1605	1269	304	
BMI, kg/m2	26.7±3.6	26.6±3.7	26.5±3.8	0.43
Waist, cm	93 ±11	92±12	91±12	0.08
Blood pressure, mmHg				
systolic	140±18	141±18	142±18	0.62
diastolic	84±11	84±11	85±11	0.61
Diabetes, n (%)	58 (3.6%)	42 (3.3%)	7 (2.3%)	0.50
CRP, mg/L	1.8 (0.8-3.9)	1.6(0.8-3.6)	1.7(0.8-3.9)	0.30
Cholesterol, mmol/L				
total	6.3±1.1	6.4±1.2	6.4±1.4	0.25
LDL	4.1±1.0	4.1±1.0	4.1±1.1	0.97
HDL	1.31±0.39	1.35±0.39	1.38±0.41	0.008
TG, mmol/L	1.8 (1.2-2.4)	1.7(1.2-2.5)	1.6(1.2-2.4)	0.28
ApoA-I, mg/dL	158±29	161±30	164±30	0.001
ApoB, mg/dL	133±32	132±32	131±33	0.627
HDL particles, nmol/L				
total	33.6±5.6	34.1±5.8	34.1±5.7	0.039
large	5.7±3.5	5.8±3.6	6.1±3.6	0.12
medium	3.3±3.0	3.5±3.1	3.4±3.2	0.26
small	24.6±4.9	24.8±5.1	24.5±5.2	0.44
HDL size, nm	8.87±0.47	8.90±0.48	8.96±0.49	0.005
HDL size (GGE), nm	8.82±0.42	8.84±0.44	8.89±0.44	0.016

Data are presented as mean (±SD) or number (percentage). Data for CRP and TG are presented as median (interquartile range). P-values for diabetes are calculated by Pearson chi-square. P-values for CRP and TG are calculated using Kruskal-Wallis tests. Other p-values are calculated by one-way ANOVA. BMI = body mass index, CRP = C-reactive protein, TG = triglycerides, ApoA-I = apolipoprotein A-I, ApoB = apolipoprotein B, GGE = gradient gel electrophoresis.

Common genetic variants in LIPG and risk of CAD

None of the SNPs we genotyped showed a significant relationship with CAD risk. For rs8093249, the OR for CAD was 0.97 per allele (95%CI 0.82-1.14, p=0.68; Fig. 2A). For rs6507931, the OR for CAD was 1.09 per allele (95%CI 0.97-1.22, p=0.16; Fig. 2B). For rs2097055, the OR was 1.06 per allele (95%CI 0.95-1.19, p=0.30; Fig. 2C), for rs2000813, it was 0.94 per allele (95%CI 0.83-1.07, p=0.33; Fig. 2D), and, finally, for rs3819166, the OR for CAD was 0.95 per allele (95%CI 0.81-1.11, p=0.50; Fig. 2E). Adjusting for individual risk factors instead of the Framingham risk score did not materially change the results (data not shown). To obtain a better estimate of the cardiovascular risk associated with rs2000813, we performed a meta-analysis of our own data combined with the published data by Jensen et al.[18] We found that, in this combined data set of 2639 CAD cases and 4843 controls, the OR for CAD was 0.96 per allele (95% CI 0.89-1.03, p=0.27), with heterozygous carriers of the minor allele having an OR for CAD of 0.96 (95% CI 0.87-1.06) and homozygous carriers having an OR for CAD of 0.91 (95%CI 0.76-1.10).

Figure 2. Odds ratios for CAD according to genotype in EPIC-Norfolk

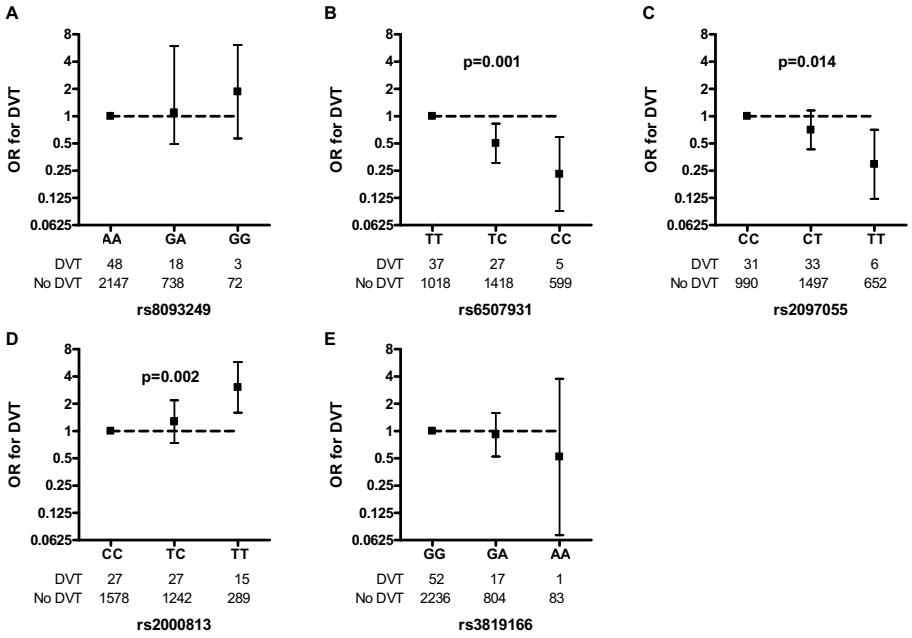


Odds ratios are adjusted for the Framingham risk score. Numbers below the charts represent the number of people per genotype, with and without subsequent CAD.

Common genetic variants in LIPG and risk of DVT

In the EPIC-Norfolk prospective CAD case-control study, 72 subjects had been diagnosed with DVT. Of the five *LIPG* tagSNPs we genotyped, three SNPs showed a significant relationship with DVT (Figure 3). For rs6507931 the per-allele OR for DVT was 2.04 (95%CI 1.40-2.98, p<0.0005; Fig. 3B), for rs2097055 the per-allele OR for DVT was 1.67 (95%CI 1.18-2.38, p=0.004; Fig. 3C), and for rs2000813, the per-allele OR for DVT was 0.60 (95%CI 0.43-0.84, p=0.003; Fig. 3D).

Figure 3. Odds ratios for DVT according to genotype in EPIC-Norfolk

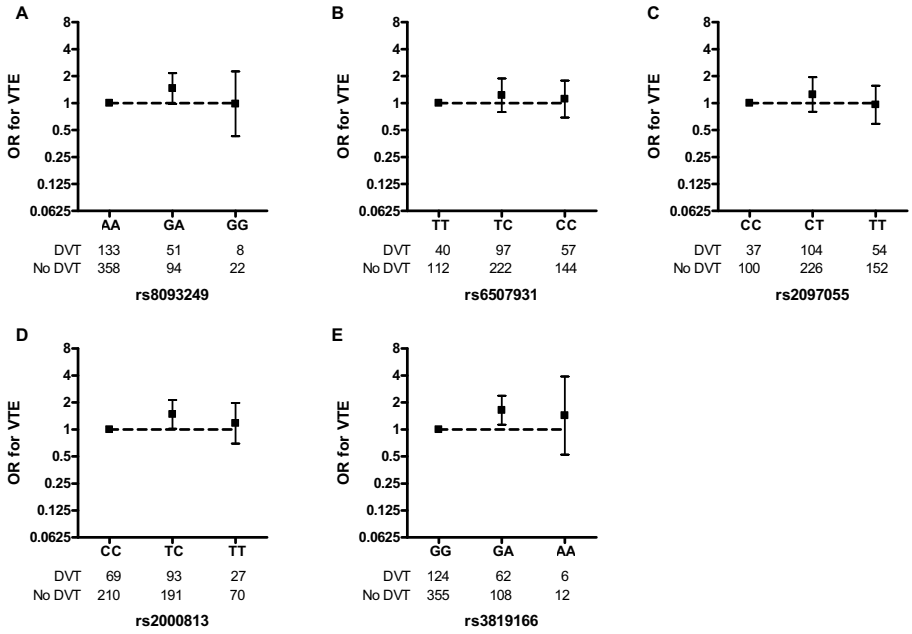


Numbers below the charts represent the number of people per genotype, with and without a history of DVT.

We set out to replicate these results in an independent study, the ACT study. Assuming true effects, the statistical power to replicate findings of this magnitude with a confidence level of 0.95 in the ACT study would be > 0.99 in each case. However, in the ACT study, no associations between these SNPs and the risk of DVT were found (Figure 4). Here, the OR for DVT was 0.96 per allele (95%CI 0.76-1.21, p=0.74; Fig 4B) for rs6507931; 1.04 per allele (95%CI 0.83-1.32, p=0.72; Fig 4C) for rs2097055 and 1.16 (95%CI 0.92-1.48, p=0.22; Fig 4D) per allele for rs2000813. We repeated the analysis after excluding all cases of provoked

DVT, potentially enriching the sample with patients with a genetic predisposition, but this did not substantially change the results (data not shown). To confirm the suitability of the ACT study to assess genetic risk factors for DVT, we genotyped Factor V Leiden (FVL) and found that this variant was associated with a per-allele OR for DVT of 3.72 (95%CI 2.26-6.13, p<0.0001).

Figure 4. Odds ratios for DVT according to genotype in the ACT study



Numbers below the charts represent the number of people per genotype, with and without DVT.

Discussion

In the present study we could determine that, out of 5 tagSNPs in the *LIPG* locus, only the minor allele of rs2000813 (p.T111I) was modestly, but consistently associated with higher HDL-C levels, higher apoA-I levels, a higher concentration of HDL particles and larger HDL size. We found no association between any of the tagSNPs and the risk of future CAD. Three SNPs, including rs2000813, were associated with DVT risk in the prospective CAD case-control study nested in the EPIC-Norfolk cohort, but these results could not be replicated in the ACT study.

Common genetic variants in LIPG and HDL metabolism

The association between the T111I variant and HDL-C levels has been evaluated in several studies, but the results have been inconsistent. Whereas some studies found higher HDL-C levels in carriers of the minor allele[9,12,13,16], others could not confirm such a relationship.[11,14,15,18] It has been proposed that a small sample size and established cardiovascular disease among study participants has limited the interpretation of some of these studies.[18] In the current study, we show in more than 3000 apparently healthy Caucasian individuals that the minor allele of T111I associates with higher HDL-C levels. Generally, a higher HDL-C is accompanied by increased apolipoprotein A-I levels and HDL particle number, as well as a larger HDL size, as is the case here. Recently, it has been shown *in vitro* that the T111I endothelial lipase variant possesses a lipolytic activity similar to that of wildtype endothelial lipase. Accordingly, in silico analysis in Polyphen suggested that the mutation is benign.[25] Therefore, the possibility should be considered that T111I is not itself a functional variant, but may be in linkage disequilibrium with another, yet unknown, variant that is functional.

Common genetic variants in LIPG and CAD risk

We found no associations between any of the *LIPG* tagSNPs and CAD risk, which is in line with the most recent findings regarding T111I from a prospective analysis in Caucasians by Jensen et al.[18] Moreover, in a meta-analysis of our data combined with those of the Jensen study, no significant association between T111I and CAD risk was observed. It has been suggested that large studies are needed to demonstrate the modest impact that single genetic variants have on complex outcomes such as CAD.[33] With the 2639 CAD cases and 4843 controls represented by the combined studies, we estimated there was 80% statistical power to exclude a relative risk of CAD of 0.93 or less. Therefore, to confidently exclude a potential 4-5% risk reduction associated with this variant, further studies would be necessary. The current null-finding is however in line with a recent string of data on genetic variants that do affect HDL-C levels but do not affect cardiovascular risk.[34,35] In fact, a direct head-to-head comparison between genetic variants influencing either plasma LDL-C or HDL-C in a study of over two thousand CAD cases and over twelve thousand controls indicated that only the former predict CAD.[36] Findings such as these have fuelled a controversy over the atheroprotective potential of HDL.

Common genetic variants in LIPG and DVT risk

Recent evidence suggests a direct link between HDL and thrombosis. HDL has been shown to inhibit several coagulation factors, such as tissue factor, factor Va, VIIIa and Xa,[37] and purified HDL has been shown to enhance the inactivation of factor Va by activated protein C (APC) and protein S, whereas purified LDL could not.[38] HDL can also scavenge anionic phospholipids, thereby abolishing their pro-coagulant properties.[39]

We found large differences in DVT risk across *LIPG* genotypes for 3 different tagSNPs in EPIC-Norfolk, suggesting a role for endothelial lipase in thrombosis susceptibility. However, we considered these results suspect for several reasons. First, the T111I variant is associated with higher HDL-C levels and earlier studies suggested a protective effect of T111I regarding CAD risk. Thus, under the assumption that DVT and CAD share a part of their aetiology, our finding that T111I was associated with *increased* DVT risk was counterintuitive. Second, such a high risk magnitude (a per-allele OR for DVT of ~1.7) in combination with such a high allele frequency (~0.3) would render this genetic risk factor more important than FVL and potentially explain a substantial part of DVT occurrence in the general population. Such a finding would be in stark contrast with the results from recent genome-wide association studies, in which no SNPs near *LIPG* were identified as risk markers for venous thromboembolism.[40,41] Finally, the EPIC-Norfolk data on DVT was self-reported, increasing the possibility of bias. For these reasons, and because we could not replicate the results in the ACT study, which was specifically designed to evaluate risk factors for DVT, we interpret the original finding regarding *LIPG* SNPs and DVT risk in EPIC-Norfolk as the result of a type I error.

In conclusion, our data support a modest association between the *LIPG* rs2000813 variant and parameters of HDL metabolism, but no association between common genetic variants in *LIPG* and CAD or DVT risk. In addition, our results reinvigorate the notion that findings of genetic association need to be replicated in independent studies.

Acknowledgements

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Supplementary Table 1. Characteristics of individuals according to *LIPG* genotype rs8093249 in EPIC-Norfolk

	rs8093249			
	AA	**AG**	**GG**	**P**
N	2195	756	75	
BMI, kg/m2	26.6±3.6	26.8±3.6	26.8±3.7	0.26
Waist, cm	92±12	94±11	93±12	0.006
Blood pressure, mmHg				
systolic	140±18	140±18	145±21	0.12
diastolic	84±11	85±12	85±13	0.80
Diabetes, n (%)	76 (3.5)	23 (3.0)	3 (4.0)	0.82
CRP, mg/L	1.7 (0.8-3.7)	1.7 (0.8-4.1)	1.8 (0.9-4.1)	0.68
Cholesterol, mmol/L				
total	6.4±1.2	6.3±1.1	6.3±1.1	0.17
LDL	4.1±1.0	4.1±1.0	4.1±0.9	0.92
HDL	1.35±0.39	1.31±0.39	1.31±0.36	0.03
TG, mmol/L	1.7 (1.2-2.5)	1.7(1.2-1.4)	1.7(1.2-2.5)	0.88
ApoA-I, mg/dL	161±30	157±29	160±26	0.009
ApoB, mg/dL	133±33	133±31	133±29	0.99
HDL particles, nmol/L				
total	34.0±5.7	33.6±5.8	33.2±5.2	0.13
large	5.8±3.6	5.6±3.5	5.6±3.6	0.18
medium	3.5±3.1	3.3±3.0	3.7±3.4	0.22
small	24.7±5.0	24.8±4.9	24.0±4.7	0.38
HDL size, nm	8.91±0.48	8.85±0.46	8.83±0.45	0.15
HDL size (GGE), nm	8.85±0.44	8.80±0.43	8.83±0.40	0.14

Data are presented as mean (±SD) or number (percentage). Data for CRP and TG are presented as median (interquartile range). P-values for diabetes are calculated by Pearson chi-square. P-values for CRP and TG are calculated using Kruskal-Wallis tests. Other p-values are calculated by one-way ANOVA. BMI = body mass index, CRP = C-reactive protein, TG = triglycerides, ApoA-I = apolipoprotein A-I, ApoB = apolipoprotein B, GGE = gradient gel electrophoresis.

Supplementary Table 2. Characteristics of individuals according to *LIPG* genotype rs6507931 in EPIC-Norfolk

	rs6507931			
	TT	**TC**	**CC**	**P**
N	1055	1508	604	
BMI, kg/m2	26.7±3.8	26.5±3.6	26.7±3.6	0.38
Waist, cm	93 ±12	92 ±12	93 ±12	0.14
Blood pressure, mmHg				
systolic	141±18	140±18	140±18	0.28
diastolic	85±12	84±11	84±11	0.12
Diabetes, n (%)	36 (3.4%)	46 (3.0%)	27 (4.5%)	0.27
CRP, mg/L	1.7 (0.8-3.9)	1.7(0.8-3.7)	1.7(0.8-3.8)	0.95
Cholesterol, mmol/L				
total	6.4±1.3	6.3±1.2	6.4±1.16	0.60
LDL	4.2±1.1	4.1±1.0	4.1±1.04	0.68
HDL	1.33±0.39	1.34±0.39	1.33±0.41	0.79
TG, mmol/L	1.7 (1.3-2.5)	1.7 (1.2-2.5)	1.7 (1.2-2.5)	0.78
ApoA-I, mg/dL	161±30	160±29	158±29	0.41
ApoB, mg/dL	133±33	132±32	133±33	0.52
HDL particles, nmol/L				
total	33.8±5.7	34.0±5.8	33.5±5.7	0.17
large	5.7±3.6	5.8±3.6	5.8±3.5	0.73
medium	3.4±3.1	3.4±3.1	3.3±3.1	0.57
small	24.8±5.0	24.8±5.0	24.5±4.7	0.41
HDL size, nm	8.88±0.47	8.89±0.48	8.88±0.48	0.87
HDL size (GGE), nm	8.84±0.43	8.84±044	8.83±0.43	0.98

Data are presented as mean (±SD) or number (percentage). Data for CRP and TG are presented as median (interquartile range). P-values for diabetes are calculated by Pearson chi-square. P-values for CRP and TG are calculated using Kruskal-Wallis tests. Other p-values are calculated by one-way ANOVA. BMI = body mass index, CRP = C-reactive protein, TG = triglycerides, ApoA-I = apolipoprotein A-I, ApoB = apolipoprotein B, GGE = gradient gel electrophoresis.

Supplementary Table 3. Characteristics of individuals according to *LIPG* genotype rs2097055 in EPIC-Norfolk

	rs2097055			
	CC	**CT**	**TT**	**P**
N	1021	1530	658	
BMI, kg/m2	26.6±3.7	26.7±3.6	26.6±3.7	0.98
Waist, cm	92±12	93±11	92±12	0.81
Blood pressure, mmHg				
systolic	141 ±19	141 ±18	140 ±18	0.22
diastolic	84 ±12	85 ±11	84 ±11	0.99
Diabetes, n (%)	37 (3.6%)	46 (3.0%)	24 (3.6%)	0.61
CRP, mg/L	1.7 (0.8-3.9)	1.8 (0.8-3.9)	1.6 (0.8-3.5)	0.09
Cholesterol, mmol/L				
total	6.4±1.3	6.4±1.2	6.3±1.1	0.96
LDL	4.1±1.0	4.1±1.0	4.2±1.0	0.93
HDL	1.33±0.39	1.34±0.39	1.32±0.39	0.32
TG, mmol/L	1.7 (1.2-2.5)	1.7 (1.2-2.5)	1.8 (1.2-2.5)	0.97
ApoA-I, mg/dL	160±29	160±30	158±29	0.14
ApoB, mg/dL	132±33	133±33	132±31	0.78
HDL particles, nmol/L				
total	33.8±5.7	34.0±5.7	33.4±5.5	0.61
large	5.7±3.6	5.8±3.6	5.7±3.4	0.90
medium	3.4±3.1	3.4±3.1	3.2±2.9	0.14
small	24.7±4.9	24.8±5.1	24.5±4.9	0.50
HDL size, nm	8.89±0.48	8.89±0.47	8.88±0.47	0.84
HDL size (GGE), nm	8.85±0.44	8.84±0.43	8.83±0.43	0.66

Data are presented as mean (±SD) or number (percentage). Data for CRP and TG are presented as median (interquartile range). P-values for diabetes are calculated by Pearson chi-square. P-values for CRP and TG are calculated using Kruskal-Wallis tests. Other p-values are calculated by one-way ANOVA. BMI = body mass index, CRP = C-reactive protein, TG = triglycerides, ApoA-I = apolipoprotein A-I, ApoB = apolipoprotein B, GGE = gradient gel electrophoresis.

Supplementary Table 4. Characteristics of individuals according to *LIPG* genotype rs3819166 in EPIC-Norfolk

	rs3819166			
	GG	**GA**	**AA**	**P**
N	2289	820	84	
BMI, kg/m2	26.6±3.7	26.7±3.5	26.9±6.5	0.73
Waist, cm	92±12	92.4±11	93±12	0.92
Blood pressure, mmHg				
systolic	140±18	141±18	142±17	0.72
diastolic	84±12	85±11	84±11	0.76
Diabetes, n (%)	82 (3.6%)	25 (3.0%)	1 (1.2%)	0.41
CRP, mg/L	1.7 (0.8-3.7)	1.7 (0.8-3.9)	1.25 (0.7-3.9)	0.66
Cholesterol, mmol/L				
total	6.4±1.2	6.3±1.2	6.3±1.1	0.70
LDL	4.1±1.0	4.1±1.0	4.2±1.0	0.84
HDL	1.35±0.4	1.32±0.37	1.27±0.45	0.08
TG, mmol/L	1.7 (1.2-2.4)	1.7 (1.2-2.5)	1.8 (1.3-2.5)	0.88
ApoA-I, mg/dL	160±30	158±27	156±30	0.12
ApoB, mg/dL	133±33	132±31	133±30	0.74
HDL particles, nmol/L				
total	34.0±5.7	33.6±5.7	32.7±5.3	0.046
large	5.8±3.6	5.6±3.4	5.7±3.7	0.34
medium	3.4±3.1	3.4±3.1	2.7±2.4	0.13
small	24.8±4.9	24.6±5. 3	24.3±4.1	0.55
HDL size, nm	8.89±0.48	8.88±0.47	8.86±0.51	0.69
HDL size (GGE), nm	8.84±4.34	8.82±0.43	8.84±0.42	0.54

Data are presented as mean (±SD) or number (percentage). Data for CRP and TG are presented as median (interquartile range). P-values for diabetes are calculated by Pearson chi-square. P-values for CRP and TG are calculated using Kruskal-Wallis tests. Other p-values are calculated by one-way ANOVA. BMI = body mass index, CRP = C-reactive protein, TG = triglycerides, ApoA-I = apolipoprotein A-I, ApoB = apolipoprotein B, GGE = gradient gel electrophoresis.

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